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COMPARATIVE EVALUATION OF GCF TOTAL ANTIOXIDANT CAPACITY IN CHRONIC PERIODONTITIS PATIENTS BEFORE AND AFTER NON-SURGICAL PERIODONTAL THERAPY

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ABSTRACT

Objectives: Periodontitis is associated with increased oxidative stress in periodontal tissue. The present study was to evaluate the Total antioxidant capacity of GCF in chronic periodontitis patients before and after non-surgical periodontal therapy.

Materials and Method: A total of 30 patients suffering from chronic periodontitis were included in the study. The patients were subjected to non-surgical periodontal therapy (NSP). Clinical parameters of Gingival Index (GI), Plaque Index (PI), and percentage of sites with bleeding on probing (BOP), probing depth (PD) and clinical attachment level (CAL) were recorded at base line and at 6 weeks. The Total antioxidant capacity in GCF was recorded at base line and 6 weeks to assess the effect of NSP on oxidative stress in periodontium.

Results: The clinical parameters showed statistically significant improvements 6 week follow up compared to baseline. The TAOC in GCF also improved significantly at 6 weeks following therapy

Conclusion: The oxidative stress in periodontal tissues was reduced following non-surgical periodontal therapy and the TAOC in can be used as a biomarker to assess the status of periodontium

KEYWORDS: Periodontitis, Total Antioxidant Capacity, Oxidative Stress & Non-Surgical Therapy

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INTRODUCTION

Periodontitis is initiated by microbial plaque which results in destruction of attachment apparatus of tooth. The mechanism of tissue destruction is through exaggerated response of host to bacteria and bacterial products **(Offenbacher, 1996)**. The first line of defense against bacterial pathogens is neutrophils, which act either by intracellular mechanisms of phagocytosis or by extracellular mechanism of increased production of ROS against bacteria. In health there exist equilibrium between ROS and antioxidants. The specific role is to remove harmful oxidants (ROS), as soon as they form, or to repair the damage caused by ROS. In a state of bacterial insults this equilibrium is disrupted leading to a state of oxidative stress **(Waddington, 2000)**. The shift of balance towards pro-oxidants not also result in direct tissue destruction but also through transcription of nuclear factor-KB and activating protein-1, which may lead to secondary tissue damage.

The oxidative stress in periodontal disease had been determined by multiple authors by assessing antioxidant levels in saliva, GCF and periodontal tissue samples (**wei D, 2010, Sang-Chul Kim et al., 2010, Chapple, 2002)** or by analyzing the products of oxidative damage of lipids, proteins and DNA. (**Akalın, 2007, EsraBaltacıoğlu, 2008).** The effect of periodontal therapy on oxidative stress has also been observed in various studies (**Sang-Chul Kim et al., 2010**, **Sukhtankar et al., 2013, Hendek, 2014).** Based on current evidence it has been postulated that oxidative stress induced in periodntium is reduced following various periodontal therapies leading to improvement in antioxidant levels. The total antioxidant capacity (TAOC), first measured by Miller in 1993, is a measure of the antioxidant capacity of all antioxidants in a biological sample and not just the antioxidant capacity of a single compound **(Miller et al., 1993**). The TAOC levels are used as biomarkers in case of chronic inflammatory conditions like Cardiovascular disease ,COPD, Diabetes Mellitus, Aging, Cancers, Stress, Renal Injury etc.

To date only few studies **(Wei D et al., 2010, Akpinar et al., 2013)** have evaluated the effect of non-surgical periodontal therapy on TAOC of GCF in chronic periodontitis patients. The purpose of the study was to evaluate GCF Total antioxidant capacity in chronic periodontitis patients before and after non-surgical periodontal therapy.

MATERIAL AND METHODS

Study Design

A total of 30 patients attending the Department of Periodontology of our institution, with moderate periodontitis were included in the study. Ethical clearance was obtained from the Hospital Ethical committee prior to the study.

Inclusion Criteria

- Patients having at least 6 sites with probing pocket depth (PPD) of >4mm and clinical attachment loss of >2mm
- Patients within the age group 18-60 years
- Patients with a minimum of 20 teeth

Exclusion Criteria

- Patients suffering from any known systemic diseases
- Patients who received any surgical or non-surgical therapy 6 months prior to start of the study
- Patients who have used any antibiotics, anti-inflammatory agents, mouthwashes, topical gels in last 3 months
- Smokers and Pan Chewers

The participation in the study was voluntary; informed consents were obtained from the patients after explaining the purpose of study and about the sample collection. The clinical parameters and GCF samples were collected at baseline prior to scaling and root planning and at 6 week post-operative follow up. Patients received supragingival scaling in first visit and were instructed to brush daily using modified bass technique. Patients were recalled after 2 weeks for subgingival scaling and root planning with respect to unresolved pockets.

Clinical Parameters

Gingival index (**Loe and Silness, 1963**), Plaque index (**Silness & Loe, 1964**), probing pocket depth (PPD), number of teeth which bled on probing (expressed as a percentage of total teeth) and clinical attachment level (CAL) were

recorded prior to NSP and at 6 weeks recall visits. Probing depth was measured from the gingival margin to the base of the pocket only in areas where the depth was more than 3mm and CAL attachment was measured from the CEJ to base of the pocket using William's periodontal probe calibrated to the nearest millimeter.

Measuring Total Anti-Oxidant Capacity (TAOC)

GCF was collected by intracrevicular method; teeth were strictly isolated with cotton rolls and gently air dried. Standardized Whatman's filter paper strips of dimensions 2x8 mm were used. A total of 6 strips were placed in each pocket, a total of 4 pockets were sampled. The filter papers were removed after 60 seconds and transferred to Eppendorf vials containing phosphate buffered saline (pH=7.4). Strips contaminated with blood were discarded and new samples were collected.

TAOC of GCF was measured using Ferric Reducing Antioxidant Power Assay (FRAP) which measures antioxidant reduction of ferric tripyridyltriazine complex to intense blue colored ferrous tripyridyltriazine, which is monitored by measuring the change in absorption at 593nm **(Benzie Iris, 1996)** .

STATISTICAL ANALYSIS

Statistical analysis was performed by SPSS 16 software. Shapiro Wilk test was performed to determine the normality of variables. Mean ± Std. deviation was used to express the variables. Paired t test was used to compare the variables which showed normal distribution (GI, PI, BOP, CAL, and PPD) and the intergroup comparisons were made by independent t-test. In case of parameters (TAOC & BOP), which didn't show normal distribution, non-parametric Wilcoxon test was used for intra group comparison and Mann-Whitney U Test was used for intergroup comparisons.

RESULTS

The mean plaque index scores at baseline was 1.65±0.19 which at 6 week follow up showed significant reduction. The gingival index showed a mean reduction of 0.57±0.03 at 6 weeks from baseline which was statistically significant. Similarly there were significant reductions in BOP (38.70±14.92 %) and probing depth (1.29±0.06 mm) at 6 week follow up. On comparing the clinical attachment change between baseline and follow up, an attachment gain of 0.80±0.18 mm was observed. The TAOC at base line was 239.00±33.7 µmol/L which improved to 292.50±40.3 with a mean change of 53.50±33.12 µmol/L which was statistically significant. **(Table 1)**

DISCUSSIONS

In the present study the change in oxidative stress in periodontium following non-surgical periodontal therapy was assessed by measuring TAOC in GCF. Oxidative stress resulting from exaggerated host response has been implicated in the pathobiology of many human diseases involving periodontitis **(Allen, 2009**). In health the ROS produced from neutrophils are neutralized by antioxidant scavenging system in humans and in this process the antioxidant levels are depleted. Similarly a reduction in oxidative stress restores the antioxidant levels representing the healthy state of tissue, in support of this concept the researchers have observed that antioxidant levels in GCF differ in periodontal disease and health (**Chapple et al 2007, Brock et al 2004, Kim et al 2010, Sukhtankar et al 2013)**.

The function of antioxidants is to protect cellular components from free radical induced damage. Antioxidant can be categorized by means of mode of action; Preventative antioxidants and chain breaking antioxidants. The preventative antioxidants function by enzymatic removal of superoxide and hydrogen peroxide e.g. superoxide dismutase enzymes

(1, 2 and 3), catalase, glutathione peroxidase, DNA repair enzymes etc. or by sequestration of divalent metal ions preventing Fenton reactions and subsequent hydroxyl radical formation, e.g. albumin, lactoferrin, transferrin, haptoglobin, ceruloplasmin, hemopexin, carotenoids, superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, uric acid, polyphenolic flavonoids etc. Scavenging (chain breaking) antioxidants are important in extracellular fluids which include Ascorbate (vitamin C), carotenoids, uric acid, polyphenols (flavenoids), bilirubin, albumin, ubiquinone, reduced glutathione etc.**(Chapple& Matthews, 2007)**

The levels of different antioxidants in saliva, serum and GCF in health and periodontal disease have been assessed in many studies **(Chapple & Matthews, 2002**, **Brock et al., 2004Wei et al.,2010)**, and the effect of non-surgical therapy on antioxidant levels also have been assessed by multiple authors **(Chappleet al., 2007, Karim et al., 2012, Yang et al, 2014).** In the present total antioxidant capacity in GCF was selected to assess the change in oxidative status of periodontium. GCF is considered to be the most appropriate sample for investigating periodontal status, because it passes through the periodontal tissues and accumulates metabolites of tissue events (**Chapple IL 2002)**. The TAOC assays evaluate the combined effectiveness of individual antioxidant species and may also account for the influence of antioxidant substances that are yet to be discovered or are difficult to assay. This also gives the capacity of biological systems to withstand oxidative attack **(Chapple & Matthews, 2007).**

CONCLUSIONS

In the present study, the total antioxidant levels increased significantly following NSP from baseline to 6 weeks corresponding to improvement in clinical parameters. Similar observations were made by various authors who demonstrated improvement in total antioxidant levels in GCF following non-surgical periodontal therapy (**Chapple et al 2007, Akpinar A et al 2013**). The improvement in TAOC levels is due removal of local factors which in turn reduced the microbial load and hence reduced generation of free radicals from neutrophils. The present study demonstrated that antioxidant profile in periodontal tissues improve following NSP and hence reducing the oxidative damage to periodontium. The present study also demonstrated that GCF TOAC can be utilized as an effective biomarker for assessing periodontal status. But the therapeutic approach of administration of both local and systemic antioxidants for reducing the oxidative tissue damage in periodontal should be evaluated through long term clinical trials.

Tables 1: Comparison of Parameters at Baseline and 6 Weeks

Parameter	Base Line	6 Weeks	Mean Change	P Value
Gingival Index†	1.63±.19	1.06±0.22	0.57±0.03	<0.001
Plaque Index†	1.65±0.19	1.09±0.20	0.55±0.03	<0.001
BOP %*	78.87±10.64	40.17±16.81	38.70±14.92	<0.001
PPD†	5.32±0.35	4.03±0.51	1.29±0.06	<0.001
CAL†	3.50±0.69	2.39±0.75	0.80±0.18	<0.001
TOAC(µmol/L)*	239.00±33.7	292.50±40.3	53.50±33.12	<0.001

†Evaluate by paired t test, *Evaluated by non-parametric Wilcoxon's test.

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